Plasmid Modification of Radiation and Chemical-mutagen Sensitivity in *Pseudomonas aeruginosa*

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(Received 7 June 1976)

SUMMARY

The R factor pMG2 protects *Pseudomonas aeruginosa* against the lethal effects of ultraviolet (u.v.) and gamma irradiation, and methyl methanesulphonate and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment. Enhanced survival occurs in strains of *uvr*⁺ *rec*⁺ (wild-type) genotype and a variety of *uvr* *rec*⁺ type mutants. No protection occurs in a *recA*-type mutant. The plasmid also enhances u.v.-induced mutagenesis. These effects appear to be due to host-cell controlled plasmid-determined DNA repair function(s). Studies on *P. aeruginosa* strains deficient in DNA polymerase I (*polA*) suggest that a plasmid-determined repair resynthesis function may be responsible for increased u.v.-survival and enhanced u.v.-mutability in pMG2-containing bacteria.

INTRODUCTION

Certain plasmids such as some colicin factors (Howarth, 1965, 1966), several enteric R factors (Drabble & Stocker, 1968; Marsh & Smith, 1969; Siccardi, 1969; MacPhee 1972, 1973*b*) and particular *Pseudomonas aeruginosa* sex factors (Krishnapillai, 1975) confer protection against the lethal effects of ultraviolet radiation on their hosts while increasing the susceptibility of the host to the mutagenic effects of ultraviolet (u.v.) light. Many u.v.-protecting plasmids (MacPhee, 1972; Krishnapillai, 1975) also improve cell survival following treatment with a number of agents capable of inducing single strand breaks in DNA (ionizing radiation, alkylating agents and thymine deprivation).

From these studies it has been suggested that u.v.-protecting plasmids may carry a gene or genes which act to increase the extent of repair of host DNA damaged by u.v. radiation, gamma (γ) radiation or methyl methanesulphonate. Further, since an intact chromosomal *recA*⁺ gene is essential for the enteric R factor, R-Utrecht, to exert its effect on the u.v. response of cells, the plasmid gene product(s) may participate in an error-prone repair pathway (MacPhee, 1973*a, b*).

The *P. aeruginosa* R factor pMG2 (Jacoby, 1974) confers resistance to gentamicin, streptomycin and sulphonamides and is a member of the compatibility group P-2. This plasmid is transmissible to various strains of *Pseudomonas* but not to *Escherichia coli* or other enteric bacteria. Other biological properties of the plasmid include resistance to inorganic and organic mercury compounds, resistance to certain DNA phages and the ability to protect bacteria against the lethal effects of u.v. radiation.

Table 1. *Bacterial strains*

Strain no.	Strain characteristics*	Reference
PAOI	prototrophic, <i>chl-2</i>	Holloway (1955)
PAO287	<i>his-52 met-28 ilv-19 arg-1</i>	Stanisich & Holloway (1969)
PU21(pMG2)	<i>ilvB112 leu-1 str-1 rif</i> FP ⁻ pMG2 (Gm Sm Su Hg Phi E79)	Jacoby (1974)
PAO287(pPL1)	<i>his-52 met-28 ilv-19 arg-1</i> pPL1 (Gm Sm Su Hg Phi-E79)	This paper
PAO2001	<i>arg-32 str-39 chl-2</i> FP ⁻	Chandler & Krishnapillai (1974)
PAO2003	<i>arg-32 str-39 chl-2 rec-2</i> FP ⁻	Chandler & Krishnapillai (1974)
TRPFI	<i>trpF1</i>	Calhoun, Pierson & Jensen (1973)
GMA848	<i>hisI1 uvr-10</i>	Kung & Lee (1973)
GMA853	<i>uvr-15</i>	Kung & Lee (1973)
GMB128	<i>gam-1</i>	Kung & Lee (1973)
GMA856	<i>hisIII5065 uvr-18</i>	Kung & Lee (1973)
GMA862	<i>hisIII5065 uvr-24</i>	Kung & Lee (1973)
GMB117	<i>mms-43</i>	Kung & Lee (1973)
GMB143	<i>mms-13</i>	Kung & Lee (1973)
GMA943	<i>thr-5431 uvr-107</i>	Kung & Lee (1973)
GMA878	<i>his-52 met-28 ilv-19 arg-1 uvr-41</i>	Kung & Lee (1973)
GMA879	<i>his-52 met-28 ilv-19 arg-1 uvr-42</i>	Kung & Lee (1973)
GMA918	<i>uvr-81</i>	Kung & Lee (1973)
GMB130	<i>gam-3</i>	Kung & Lee (1973)
GMB110	<i>mms-3</i>	Kung & Lee (1973)
GMB147	<i>hcr-10</i>	Kung & Lee (1973)
GMB111	<i>polA2</i>	Lehrbach <i>et al.</i> (1976)
GMB112	<i>polA3</i>	Lehrbach <i>et al.</i> (1976)
GMB123	<i>polA4</i>	Lehrbach <i>et al.</i> (1976)

* Antibiotic resistances: *chl*, chloramphenicol; Gm, gentamicin; Sm or *str*, streptomycin; Su, sulphonamide; *rif*, rifampin. Hg, resistance to mercuric ion. Phi, interference with phage propagation.

This paper reports a study of the u.v.-protecting ability of pMG2 in a variety of radiation-sensitive and chemical-mutagen-sensitive mutants of *Pseudomonas aeruginosa* (ATCC15692) (Kung & Lee, 1973). Several of the mutant strains tested are deficient in a DNA polymerase I type activity (Lehrbach, Kung & Lee, 1976). A recombination-deficient (*rec*) mutant of *P. aeruginosa* (PAO2003) (Chandler & Krishnapillai, 1974), phenotypically similar to the *recA* mutants of *E. coli* K12 (Clark & Margulies, 1965), has also been included in this study.

METHODS

Bacteria. The standard wild-type strain is *P. aeruginosa* PAOI (ATCC15692) (Holloway, 1955). Strains used in this study are listed in Table 1.

Media. Media and the sources of chemicals have been described previously (Kung & Lee, 1973). Antibiotics were added at the following concentrations ($\mu\text{g ml}^{-1}$): gentamicin, 20; streptomycin, 200; rifampin, 100. For selecting or scoring Hg²⁺ resistance, HgCl₂ was added to appropriately supplemented minimal medium to a final concentration of 0.5 mM.

General culture procedures and mutagenesis. These techniques have been described previously (Kung & Lee, 1973).

Conjugational transfer of R factor. Exponentially growing cultures of donor and recipient were mixed in a ratio of 1:10, respectively, in 3 ml nutrient broth containing 4 mg KNO₃ ml⁻¹ (Loutit, Pearce & Marinus, 1968). The mating mixture was incubated overnight without shaking in a sterile glass Petri plate at 37 °C. Recipients harbouring the R factor were

selected by appropriate genotype and acquired resistance to gentamicin, streptomycin and Hg^{2+} ions (sulphonamide resistance was not tested).

γ - and u.v.-irradiation. This was done as previously described by Kung & Lee (1973).

Treatment with alkylating agents. Determination of methyl methanesulphonate (MMS) sensitivity has been described previously (Lehrbach *et al.*, 1976). To determine survival in wild-type strains (PAO1 and PAO2001), the bacteria were suspended in Tris/sodium/magnesium buffer (TNM) containing 0.4 % (v/v) MMS. Survival of chemical-mutagen-sensitive strains was determined in 0.2 % (v/v) MMS. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) sensitivity was determined by resuspending 1 ml stationary-phase bacteria in 5 ml TNM containing 20 μg NTG ml^{-1} . The mixture was held at 25 °C and samples were removed at intervals, diluted and plated on nutrient agar plates.

Host-cell reactivation (hcr) experiments. Hcr experiments for MMS and u.v. radiation have been described previously (Lehrbach *et al.*, 1976).

U.v.-mutagenesis. To determine the frequency of u.v.-induced mutations, the yield of Trp^+ back mutations of strain TRPFI following various u.v. radiation doses was scored. Samples (0.1 ml) of irradiated and control suspensions were spread on minimal agar medium supplemented with 2.5 % (v/v) nutrient broth. Survival following each u.v. radiation dose was determined by plating on nutrient agar.

Assay for DNA polymerase activity. Bacteria to be used in assays were grown on the surface of solid media (nutrient agar) at 37 °C for 24 h. They were washed from the surface with saline, centrifuged and resuspended in 0.1 M-Tris/0.01 M- MgSO_4 (pH 6.6) at a concentration of about 10^{11} bacteria ml^{-1} . Crude extracts of these suspensions were prepared by sonication in a 150 W M.S.E. ultrasonic disintegrator and assayed for DNA polymerase activity by the method of De Lucia & Cairns (1969).

RESULTS

Effect of pMG2 on radiation sensitivity

The R factor pMG2 was transferred to selected members of the defined phenotypic classes of radiation-sensitive and chemical-mutagen-sensitive mutants of *Pseudomonas aeruginosa* (Kung & Lee, 1973). The effects of pMG2 on survival following u.v.- and γ -irradiation are summarized qualitatively in Table 2.

Typical survival curves for strains with and without the R factor are given in Fig. 1. These studies indicate: (1) pMG2 is capable of enhancing host survival following u.v.- and γ -irradiation in wild-type and all classes of repair-deficient strains except a recombination-deficient mutant PAO2003. (2) The protection effect is on the shoulder of the survival curve, the final slope remaining unchanged. This is similar to the effect of ColI (Howarth, 1965) and certain enteric R factors (Siccardi, 1969).

Effect of pMG2 on survival following MMS and NTG treatment

MMS and NTG are thought to cause single strand breaks in DNA. The repair of single strand breaks, whether due to spontaneous hydrolysis of the methylated base, or to enzymically mediated excision (Lawley, 1974), is effectively identical to the repair of strand breaks caused by ionizing radiation. Table 2 and Fig. 2(a) show that the protecting effect of pMG2 extends to treatment with MMS in phenotypic groups 3 to 7, 11 and wild-type. Qualitatively similar results were obtained for treatment with NTG (Table 2 and Fig. 2b).

Fig. 2. Survival in strains with and without R factor pMG2 following: (a) MMS treatment; (b) NTG treatment. ■, PAO1(pMG2); □, PAO1; ●, GMB117(pMG2); ○, GMB117.

Influence of pMG2 in host-cell reactivation (hcr) mutants

Protection against u.v. radiation by pMG2 could conceivably be due to a plasmid function which may participate in the excision of pyrimidine dimers. If so, one might expect pMG2 to (i) restore the u.v.-sensitivity of excision-deficient mutants to nearly wild-type levels, (ii) restore the capacity to host-cell reactivate u.v.-irradiated phage.

Since cells carrying pMG2 fail to plate phage E79 (Jacoby, 1974) it was necessary to isolate a mutant of the R factor which does not affect the phage plating efficiency of the bacterial host strain. The derived R factor pPL1 is able to transfer the drug-resistance properties and confer protection against u.v. radiation and MMS damage on its host, while cells harbouring pPL1 remain sensitive to phage E79.

Both pMG2 and pPL1 provide some u.v.-protection to a typical excision-deficient (*hcr*) strain GMA918, although they do not restore this strain to the wild-type level of survival (Fig. 1a) – the result which might have been expected if the protecting effect was due to a plasmid product functionally similar to a u.v.-repair enzyme deficient in an *hcr* mutant.

Wild-type PAO1 and strain GMA918 were tested for their ability to host-cell reactivate u.v.-irradiated phage E79 in the presence of pPL1. pPL1 did not improve the *hcr* ability of either strain (Fig. 3).

Effect of rec and pol host mutations on the u.v.-protecting property of pMG2

Studies using the enteric plasmid R-Utrecht have shown that the u.v.-protecting effect depends upon a functional *recA* gene present in the host cells (MacPhee, 1973b). Recently mutants have been isolated in *P. aeruginosa* (Chandler & Krishnapillai, 1974) which are phenotypically similar to *recA* mutants of *E. coli*. The recombination-deficient *recA*-type mutant PAO2003 carrying pMG2 showed no change in survival following u.v.-irradiation or MMS treatment (Fig. 4). A similar u.v. response has previously been shown by Jacoby (1974) using a u.v.-irradiation hypersensitive *rec* strain JC9010.

Fig. 3. Host-cell reactivation of u.v.-irradiated phage E79 on strains: ■, PAO1(pPL1); □, PAO1; ●, GMA918(pPL1); ○, GMA918.

Fig. 4. Survival of *rec* and *pol* strains with and without pMG2 following: (a) u.v.-irradiation; (b) MMS treatment. ■, PAO2001(pMG2); □, PAO2001; ▲, GMB112(pMG2); △, GMB112; ●, PAO2003-(pMG2); ○, PAO2003.

The biochemical characterization of several *pol* mutants of *P. aeruginosa* (Lehrbach *et al.*, 1976) also allows the study of the behaviour of pMG2 in bacteria with a *pol* background. Strain GMB112 is deficient in the enzyme DNA polymerase I and shows increased sensitivity to u.v. and γ radiation, and MMS and NTG as well as having reduced ability to reactivate phage E79 treated with u.v. radiation or MMS. GMB112 carrying the R factor pMG2 or

Fig. 5. Host-cell reactivation of (a) u.v.-irradiated, (b) MMS treated phage E79 on strains: □, PAO1; ▲, GMB112(pPL1); △, GMB112.

Table 3. Effect of pMG2 on u.v.-induction of *Trp*⁺ revertants in strain TRPFI

Numbers show the frequency of *Trp*⁺ revertants per 10⁷ survivors (corrected for spontaneous frequency); numbers in parentheses show percentage survival.

Strain	Spontaneous frequency	Induced frequency at u.v. dose (J m ⁻²):		
		10	20	30
TRPFI	5	10 (70)	31 (10)	43 (0.8)
TRPFI(pMG2)	18	280 (100)	360 (96)	560 (80)

pPL1 was only slightly protected against the lethal effects of u.v.-irradiation and MMS-treatment (Fig. 4). Further tests with GMB112 carrying pPL1 showed that this plasmid increases the hcr capacity of strain GMB112 for both u.v.-irradiated and MMS-treated phage E79 to a level approximately equal to that of the wild-type parent strain PAO1 (Fig. 5). Plasmid pPL1 had no detectable effect on hcr capacity in the wild-type parent (Fig. 3).

Influence of pMG2 on u.v.-induced mutation

The previous results suggest that pMG2 provides hosts deficient in the enzyme DNA polymerase I with a plasmid-borne product which can participate in the repair of single strand gaps. Protection by pMG2 depends on a *RecA*⁺ phenotype and since error-prone repair processes depend on an intact *recA*⁺ gene (Witkin, 1969) we determined whether pMG2 had an effect on the yields of induced mutations. The R factor was transferred to the tryptophan auxotroph TRPFI (Calhoun *et al.*, 1973). Back mutation to prototrophy was tested following exposure to low doses of u.v. radiation. pMG2 substantially increased the mutation frequency induced by u.v.-irradiation and also caused a three- to fourfold increase

Fig. 6. Assay of polymerase activity of strains: $\square$, PAOI; $\bullet$, GMB112(pMG2); $\circ$, GMB112.

in spontaneous back mutation (Table 3). Similar results were obtained for the mutant R factor pPL1.

DNA polymerase activity associated with pMG2

Studies with the u.v.-protecting plasmid R-Utrecht (MacPhee, 1974) have presented evidence of a plasmid-borne DNA polymerase function resembling the chromosomal DNA polymerase I (i.e. readily assayable in crude sonicated extracts).

DNA polymerase assays were performed with strain GMB112 harbouring pMG2. Crude extracts of GMB112 showed no detectable polymerizing activity whereas pMG2 derivatives showed a significant increase in polymerase activity (Fig. 6). This activity was about 50 % of that in extracts of wild-type bacteria assayed under similar conditions (see Methods).

DISCUSSION

The results indicate that the R factor pMG2 protects *P. aeruginosa* against the lethal effects of u.v. and γ radiation, and MMS and NTG. Enhanced survival occurs in strains of *uvr⁺ rec⁺* (wild-type) genotype and *uvr rec⁺* type mutants (*uvr*-type mutants; phenotypic groups 1 to 6, 8, 9 and 12, Table 2), suggesting that the plasmid gene product(s) may contribute to the dark repair of u.v., γ , MMS and NTG damaged host DNA.

The presence of the plasmid fails to improve the host-cell reactivation capacity of an *hcr*-type mutant (GMA918) or wild-type strain (PAOI), with respect to u.v.-irradiated phage E79. However, pMG2 significantly enhances the ability of strain GMB112, deficient in a DNA polymerase I type activity, to host-cell reactivate u.v.-irradiated and MMS-treated phage E79. These results suggest that the plasmid gene product(s) is not involved in the excision step of excision repair, but may participate in the repair of single strand gaps produced in phage DNA following excision of u.v.-induced pyrimidine dimers or as a result of MMS-induced damage.

In wild-type cells (*polA⁺*) of *E. coli*, DNA polymerase I is mainly responsible for carrying out repair replication or repair resynthesis involved in excision repair of u.v.-damage (Monk, Peacey & Gross, 1971). If the plasmid gene product(s) which may be responsible for the enhanced u.v.-survival of wild-type cells of *P. aeruginosa* can perform a repair resynthesis

similar to the chromosomal-coded polymerase I enzyme then pMG2 would not be expected to increase the u.v.-survival of *hcr* mutants (presumably unable to excise pyrimidine dimers). In the *P. aeruginosa hcr* mutants reported here there is increased survival following u.v.-irradiation (Table 2).

We have attempted to assess the plasmid-determined repair resynthesis activity by means of a crude extract assay system used for detection of DNA polymerase I activity (De Lucia & Cairns, 1969). Previous results (Lehrbach *et al.*, 1976) indicated that crude extracts of strain GMB112 contained little or no detectable DNA polymerase activity (less than 2 % of normal wild-type activity). However, pMG2 derivatives of GMB112 showed a significant increase in polymerizing activity.

Since the u.v.-protecting ability of pMG2 is dependent on an intact *recA* type gene product and the plasmid enhances the level of u.v.-induced mutation (detected by back mutation to *trp*⁺ in strain TRPFI), it is possible that the plasmid-determined repair resynthesis is involved in an error-prone repair pathway. Therefore it may be the involvement of the plasmid gene product in this error-prone repair pathway that accounts for the increased u.v.-survival of *uvr*-type mutants, *pol*-type mutants and wild-type strains containing the plasmid.

One hypothesis considered is that the plasmid pMG2 specifies a DNA polymerase type product which is able to take part in the repair of u.v., γ , MMS and NTG damaged DNA and is more prone to introduce errors during the filling of DNA strand gaps (i.e. certain gaps produced during post-replicative repair and certain excision gaps). Such a function would account for the increased *hcr* capacity in a polymerase-defective strain and both the u.v.-protection and enhanced u.v.-mutability of cells harbouring pMG2. The possibility of such an enzyme as an inducible function in u.v.-mutagenesis in *E. coli* has been suggested (Witkin, 1974). Alternatively several plasmid genes may be involved in producing the u.v.-protecting effect and enhanced mutability.

R factor mutants have been isolated which have lost the ability to enhance u.v.-mutability. Preliminary data show that these mutants have also lost the ability to protect cells against the lethal effects of u.v. radiation. Crude extracts of GMB112 containing one of these mutant R factors showed no detectable increase in polymerizing activity over that of polymerase-defective strain GMB112 (unpublished data).

The results therefore suggest that pMG2 codes for one gene product which participates in an error-prone repair pathway of *P. aeruginosa*. This gene product, detected biochemically by the increased polymerase activity in the polymerase-defective strain GMB112, is responsible for the u.v.-protection and enhanced mutability observed in plasmid-containing bacteria.

This work was supported by a Research and Training grant from the Australian Institute of Nuclear Science and Engineering. We would like to thank Dr F. P. Imray for her comments during the preparation of the manuscript and Margaret Sawyer for her help. G. A. Jacoby was supported by a grant from the National Sciences Foundation. Gentamicin sulphate (Schering) was a gift from Essex Laboratories.

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